

# *Spore Size Comparison Between Several Bacillus Species*

Ruben O. Zandomeni<sup>1</sup>, Joseph E. Fitzgibbon<sup>2</sup>, Monica Carrera<sup>1</sup>, Edward Stuebing<sup>2</sup>, James E. Rogers<sup>2</sup>, and Jose-Luis Sagripanti<sup>2</sup>

<sup>1</sup>GeoCenters, Inc. and <sup>2</sup>RDECOM, Edgewood Chemical and Biological Center, US Army, Aberdeen Proving Ground, MD 21010



<b>Report Documentation Page</b>			Form Approved OMB No. 0704-0188	
<p>Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.</p>				
1. REPORT DATE <b>01 OCT 2005</b>	2. REPORT TYPE <b>N/A</b>	3. DATES COVERED <b>-</b>		
4. TITLE AND SUBTITLE <b>Spore Size Comparison Between Several Bacillus Species</b>			5a. CONTRACT NUMBER	
			5b. GRANT NUMBER	
			5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)			5d. PROJECT NUMBER	
			5e. TASK NUMBER	
			5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) <b>GeoCenters, Inc.</b>			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)			10. SPONSOR/MONITOR'S ACRONYM(S)	
			11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT <b>Approved for public release, distribution unlimited</b>				
13. SUPPLEMENTARY NOTES <b>See also ADM001851, Proceedings of the 2003 Joint Service Scientific Conference on Chemical &amp; Biological Defense Research, 17-20 November 2003. , The original document contains color images.</b>				
14. ABSTRACT				
15. SUBJECT TERMS				
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT <b>UU</b>	18. NUMBER OF PAGES <b>31</b>
a. REPORT <b>unclassified</b>	b. ABSTRACT <b>unclassified</b>	c. THIS PAGE <b>unclassified</b>	19a. NAME OF RESPONSIBLE PERSON	

# **Introduction**

- **GOALS** - Better understand properties of simulants vs. agents. Provide data to help choose appropriate simulants and surrogates for specific tasks.
- **METHOD** - Compare properties of simulants to those of the agents.
- **FACILITY**  
**Microbiology**  
**Team**  
**BSL3**  
**Laboratory**



# **Properties (Non-Medical)**

- Spore Size – length, width, aspect ratio
- Spore Density
- Effect of decon agents
- Fluorescence spectra
- Effect of UV radiation

# Spore Size

- Important to design and development of samplers and detectors
- Published spore sizes - spores produced under different conditions for each species without extensive comparisons, size distributions or ranges.
- Systematic comparison of the size of *B.anthracis* spores to size of other *Bacillus* spores (simulants/surrogates) - all spores produced under the same conditions.

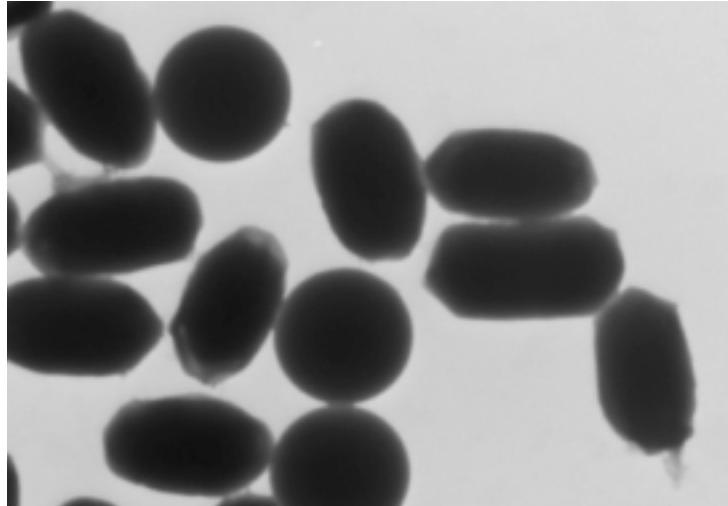
# Methods

- Prepared spores of *B.anthracis* and other *Bacillus* species, including some common simulants/surrogates.
- Spores were fixed, negatively stained and imaged by TEM.
- Measurements of 100 spores (length, width, aspect ratio).
- Produced distributions.

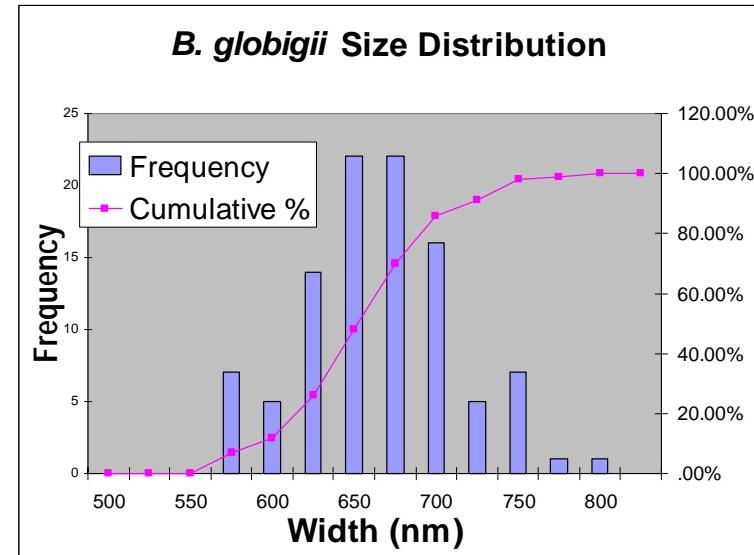
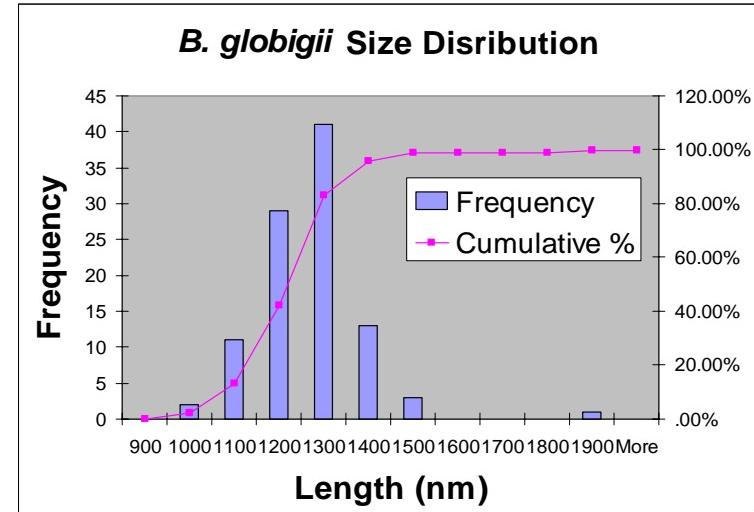


ECBC

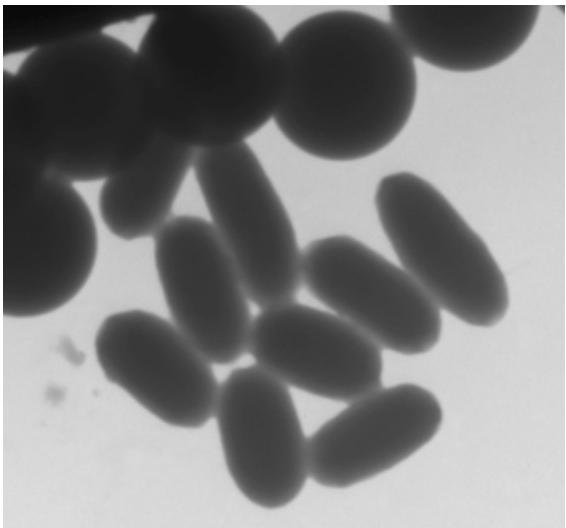
# *B. globigii* SB512 (*B. atrophaeus*)



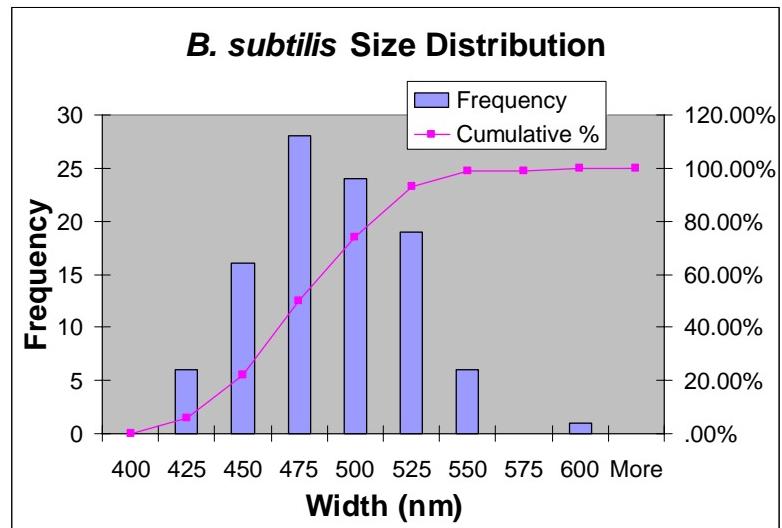
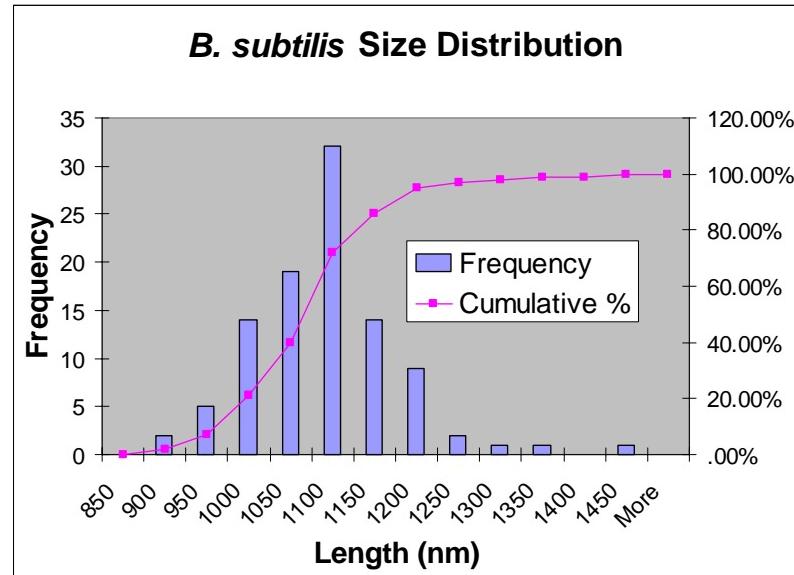
- Length  
**Mean  $1.22 \pm 0.12 \mu\text{m}$**   
**Range  $0.91$ - $1.90 \mu\text{m}$**
- Width  
**Mean  $0.65 \pm 0.05 \mu\text{m}$**   
**Range  $0.56$ - $0.80 \mu\text{m}$**



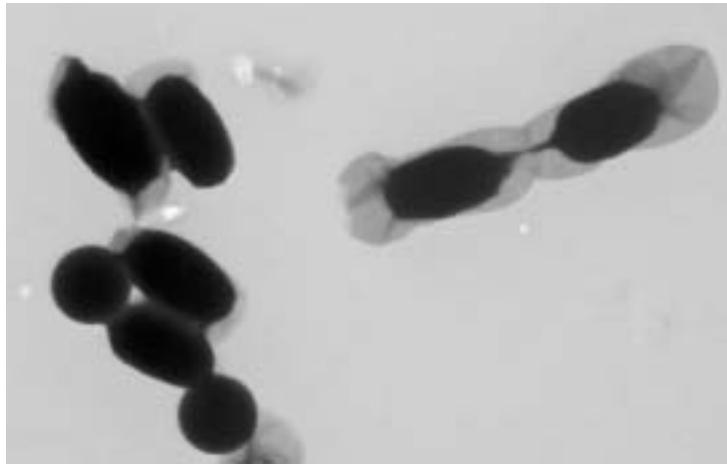
# *B. subtilis* 1031



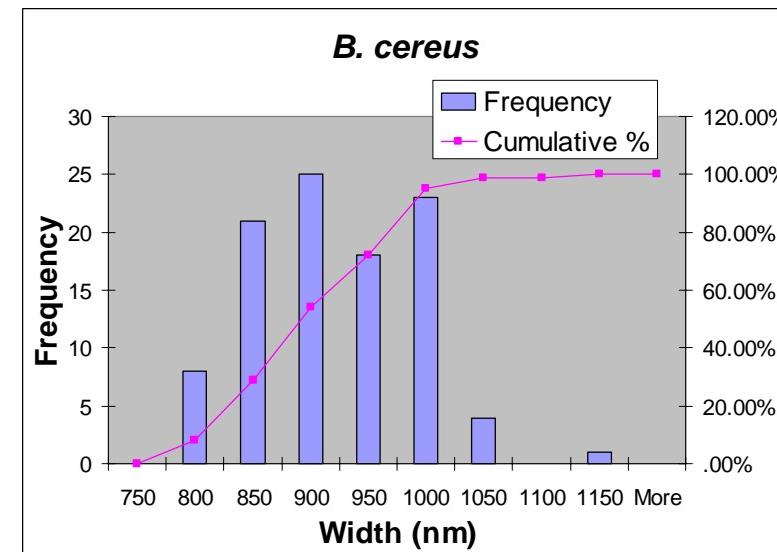
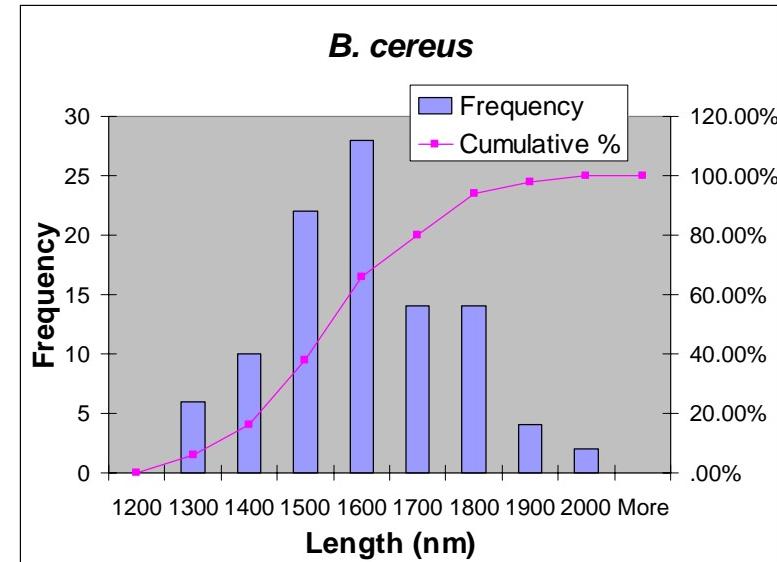
- **Length**  
**Mean  $1.07 \pm 0.09 \mu\text{m}$**   
**Range  $0.87$ - $1.41 \mu\text{m}$**
- **Width**  
**Mean  $0.48 \pm 0.03 \mu\text{m}$**   
**Range  $0.41$ - $0.58 \mu\text{m}$**



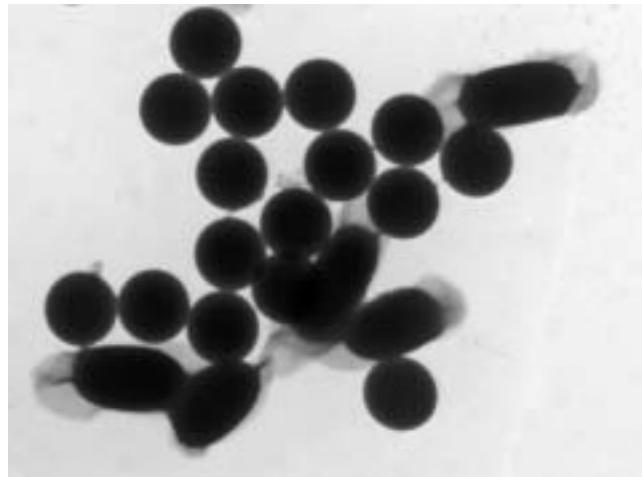
# *B. cereus* ATCC 10702



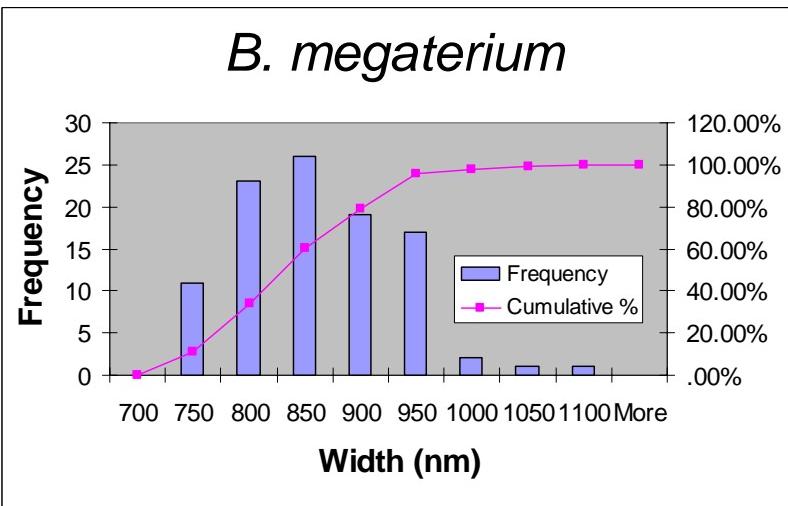
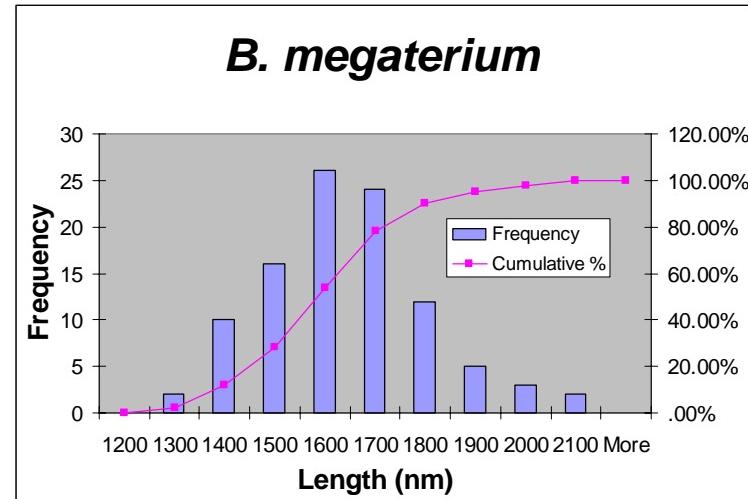
- **Length**  
**Mean  $1.55 \pm 0.16$   $\mu\text{m}$**   
**Range  $1.21$ - $2.00$   $\mu\text{m}$**
- **Width**  
**Mean  $0.90 \pm 0.07$   $\mu\text{m}$**   
**Range  $0.76$ - $1.14$   $\mu\text{m}$**



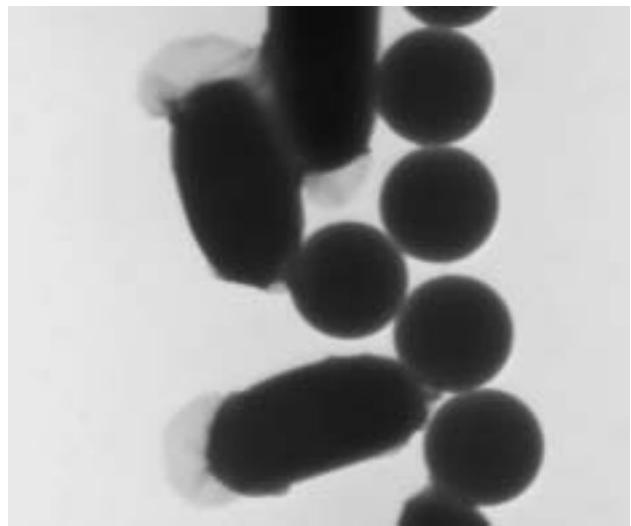
# *B. megaterium* WW-15-4900



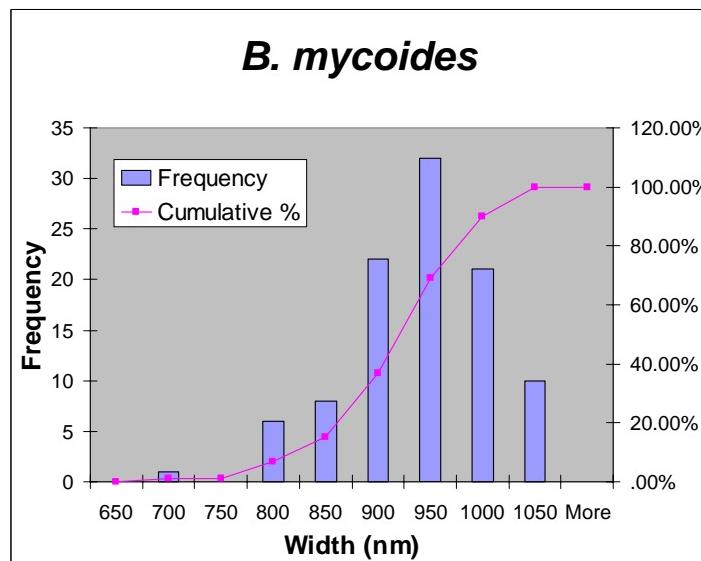
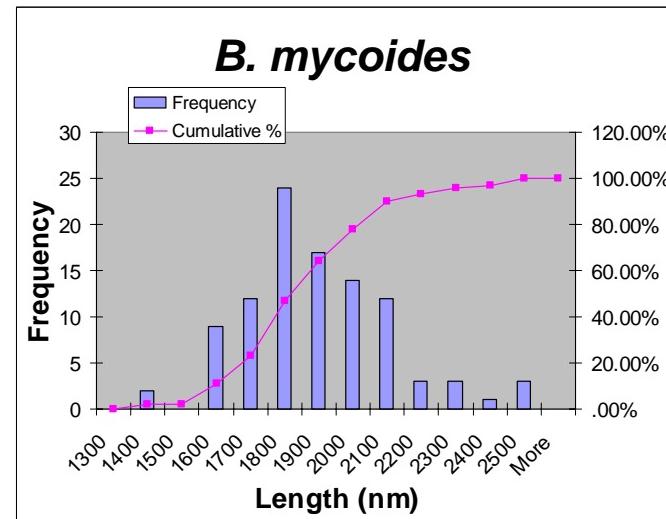
- **Length**  
Mean  **$1.60 \pm 0.16 \mu\text{m}$**   
Range  **$1.28\text{-}2.04 \mu\text{m}$**
- **Width**  
Mean  **$0.84 \pm 0.07 \mu\text{m}$**   
Range  **$0.70\text{-}1.05 \mu\text{m}$**



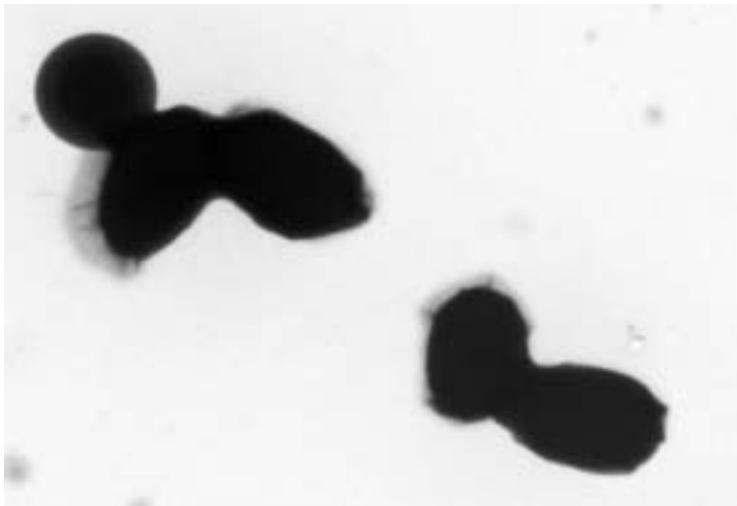
# *B. mycoides* ATCC 10206



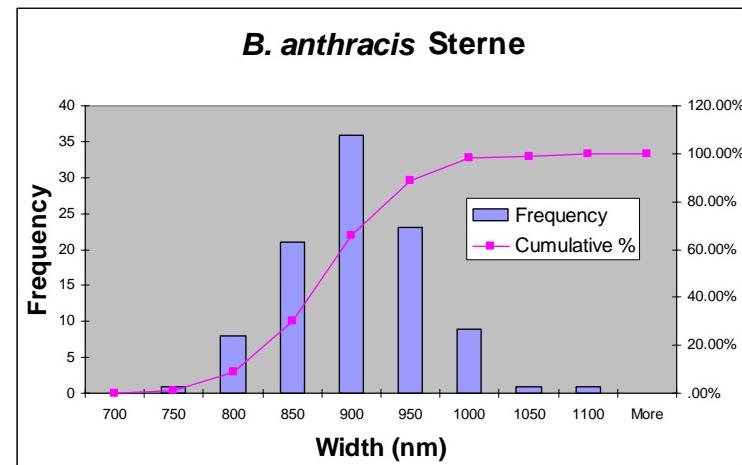
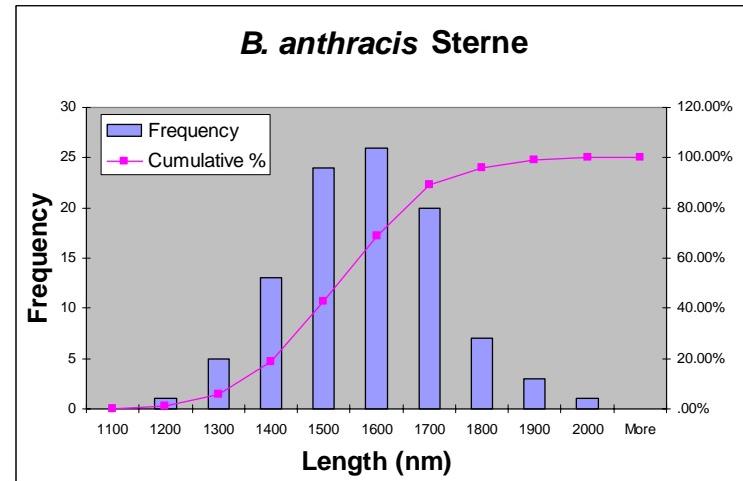
- **Length**  
**Mean  $1.85 \pm 0.21 \mu\text{m}$**   
**Range  $1.33\text{-}2.44 \mu\text{m}$**
- **Width**  
**Mean  $0.91 \pm 0.07 \mu\text{m}$**   
**Range  $0.65\text{-}1.04 \mu\text{m}$**



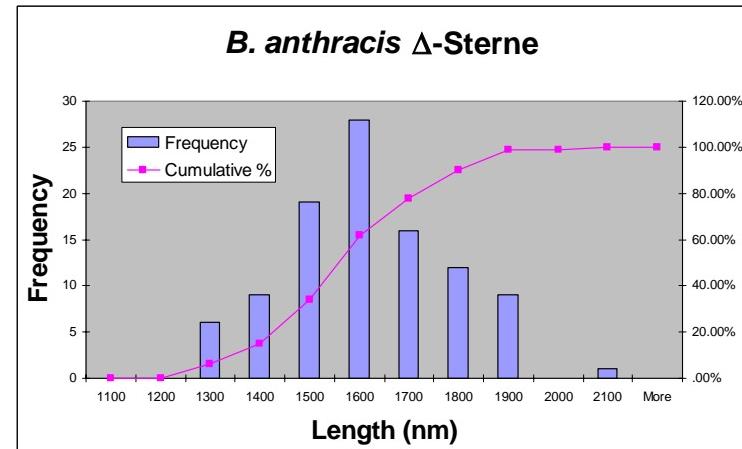
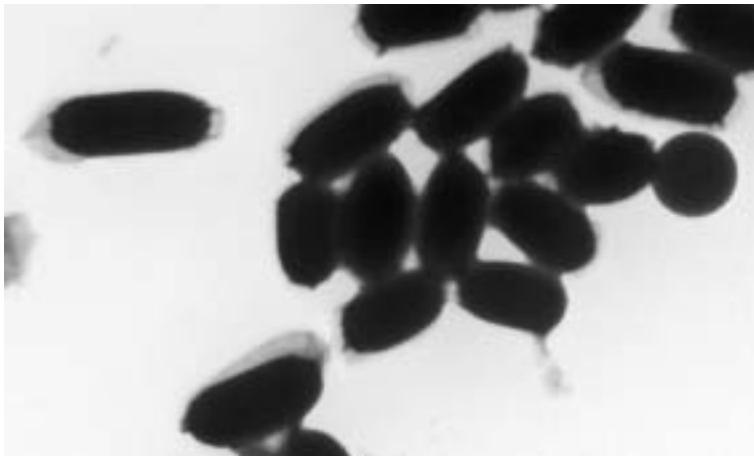
# *B. anthracis* (Sterne)



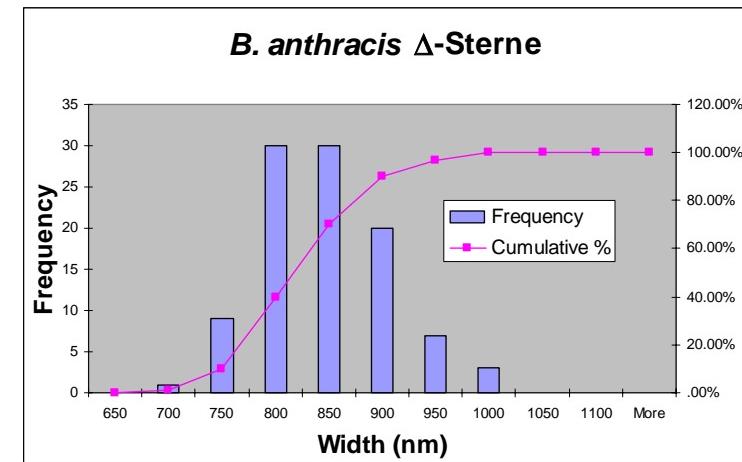
- **Length**  
**Mean  $1.53 \pm 0.15 \mu\text{m}$**   
**Range  $1.19\text{-}1.92 \mu\text{m}$**
- **Width**  
**Mean  $0.88 \pm 0.06 \mu\text{m}$**   
**Range  $0.71\text{-}1.09 \mu\text{m}$**



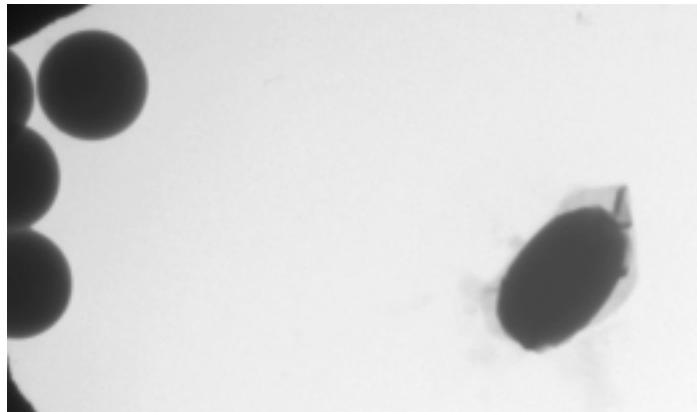
# *B. anthracis* ( $\Delta$ -Sterne)



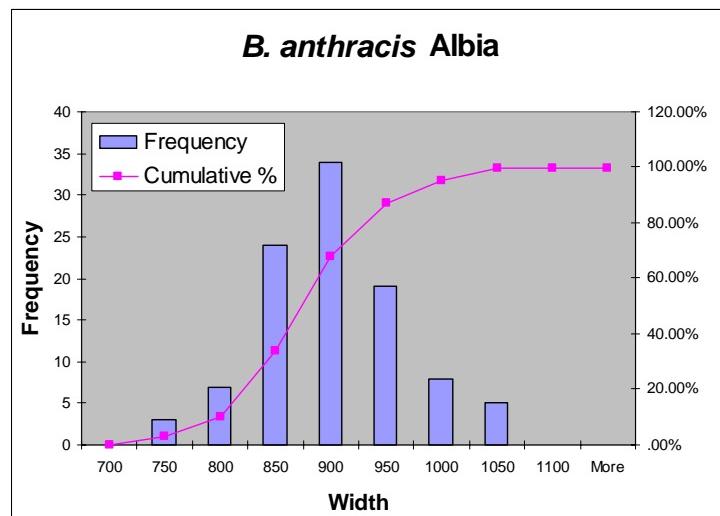
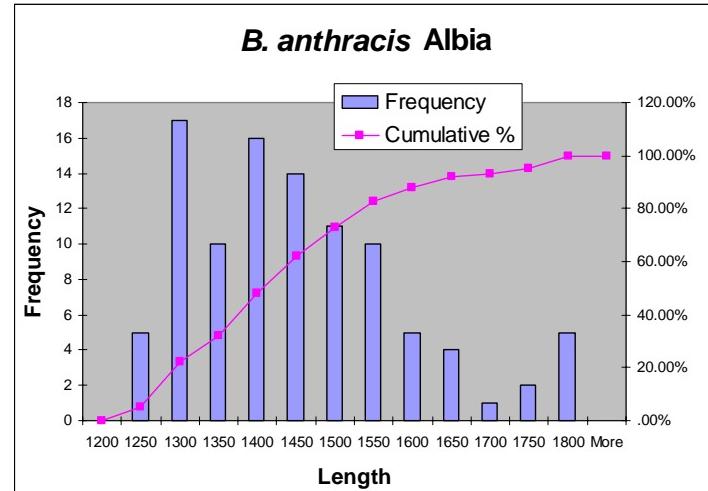
- **Length**  
Mean  $1.56 \pm 0.16 \mu\text{m}$   
Range  $1.23$ - $2.05 \mu\text{m}$
- **Width**  
Mean  $0.82 \pm 0.06 \mu\text{m}$   
Range  $0.68$ - $0.98 \mu\text{m}$



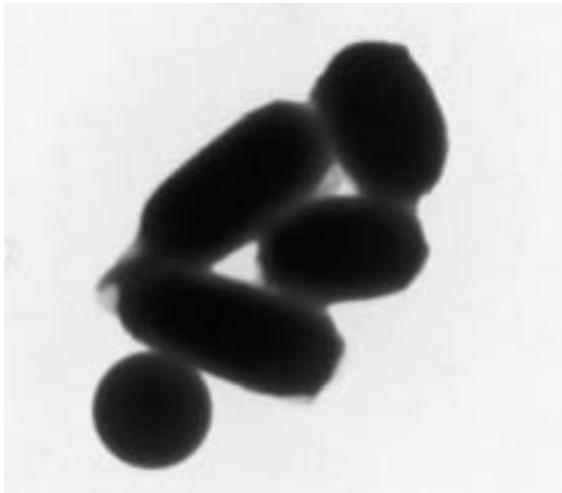
# *B. anthracis* (Albia)



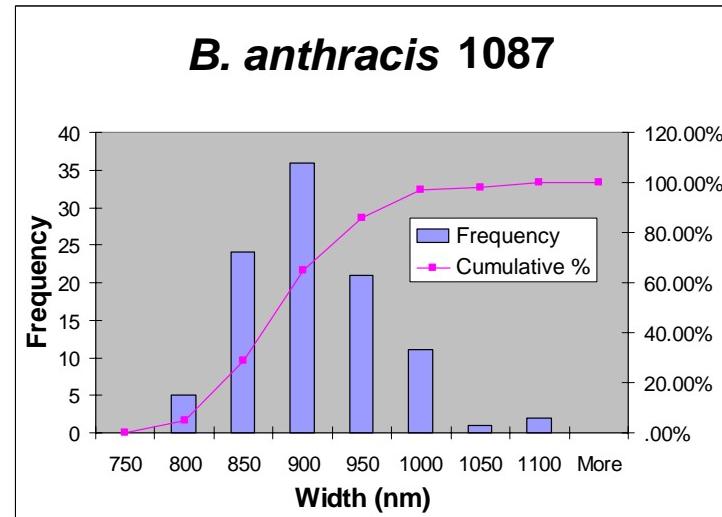
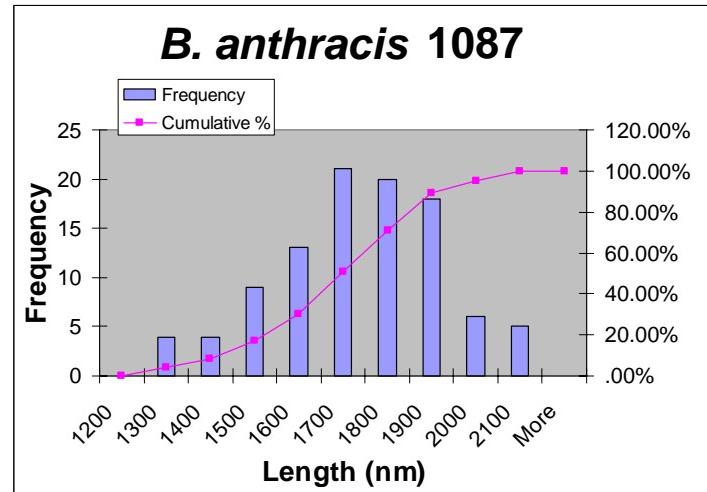
- **Length**  
Mean  $1.43 \pm 0.14 \mu\text{m}$   
Range  $1.22$ - $1.79 \mu\text{m}$
- **Width**  
Mean  $0.87 \pm 0.07 \mu\text{m}$   
Range  $0.71$ - $1.03 \mu\text{m}$



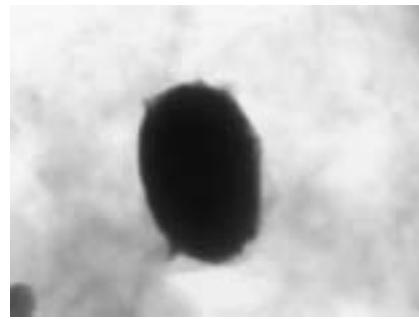
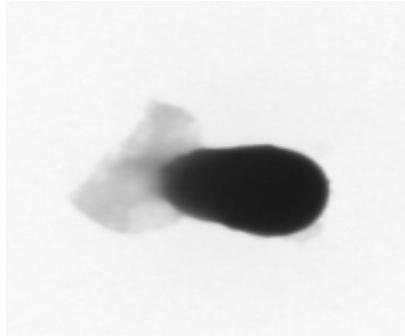
# *B. anthracis* (NCTC 1087)



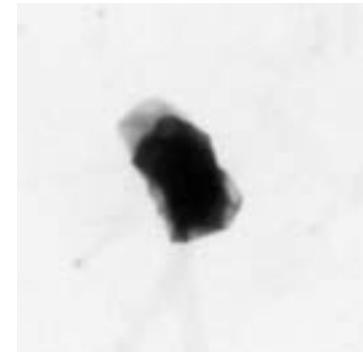
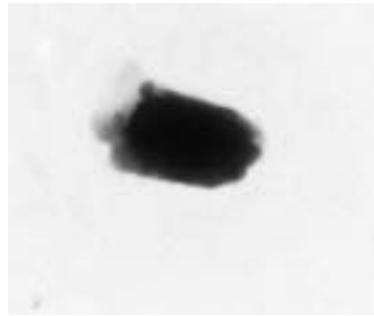
- **Length**  
Mean  $1.68 \pm 0.19 \mu\text{m}$   
Range  $1.23$ - $2.08 \mu\text{m}$
- **Width**  
Mean  $0.89 \pm 0.06 \mu\text{m}$   
Range  $0.76$ - $1.09 \mu\text{m}$



# *B. anthracis* (NCTC 1928)

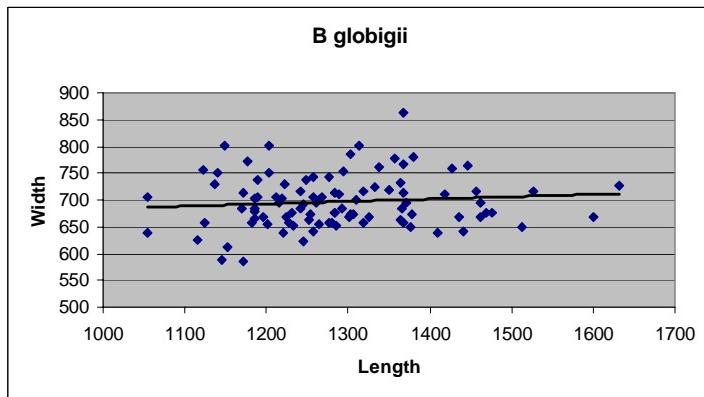


# *B. anthracis* (LA1)



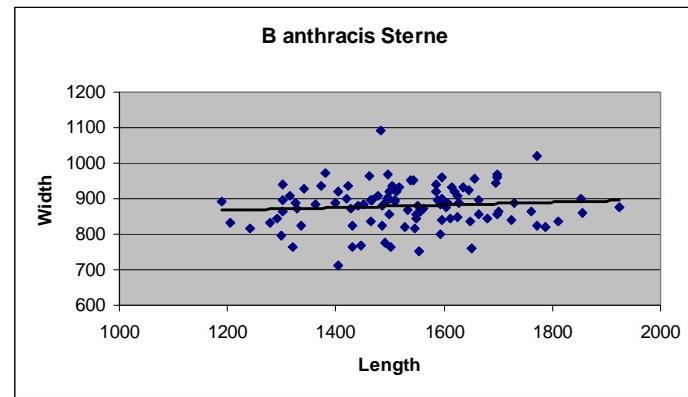
**ECBC**

# Aspect Ratio (L/W) of Bacillus spores



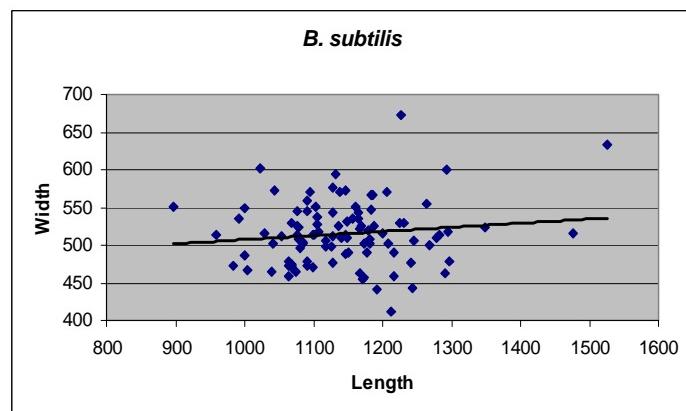
*B. globigii*

Mean **1.85 +/- 0.19**  
Range **1.43-2.39**



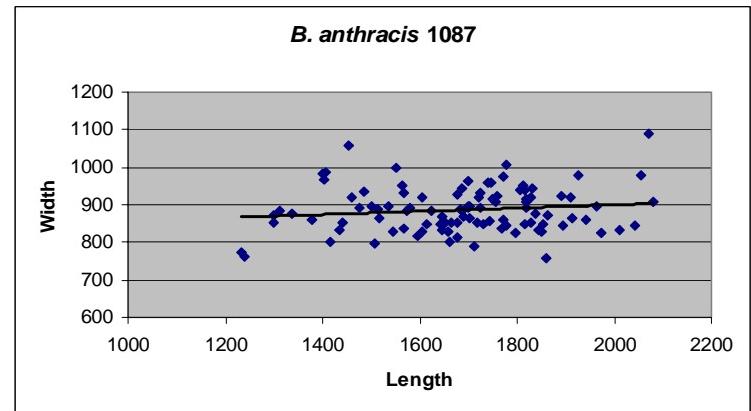
*B. a. Sterne*

Mean **1.75 +/- 0.20**  
Range **1.33-2.20**



*B. subtilis*

Mean **2.23 +/- 0.25**  
Range **1.63-2.95**



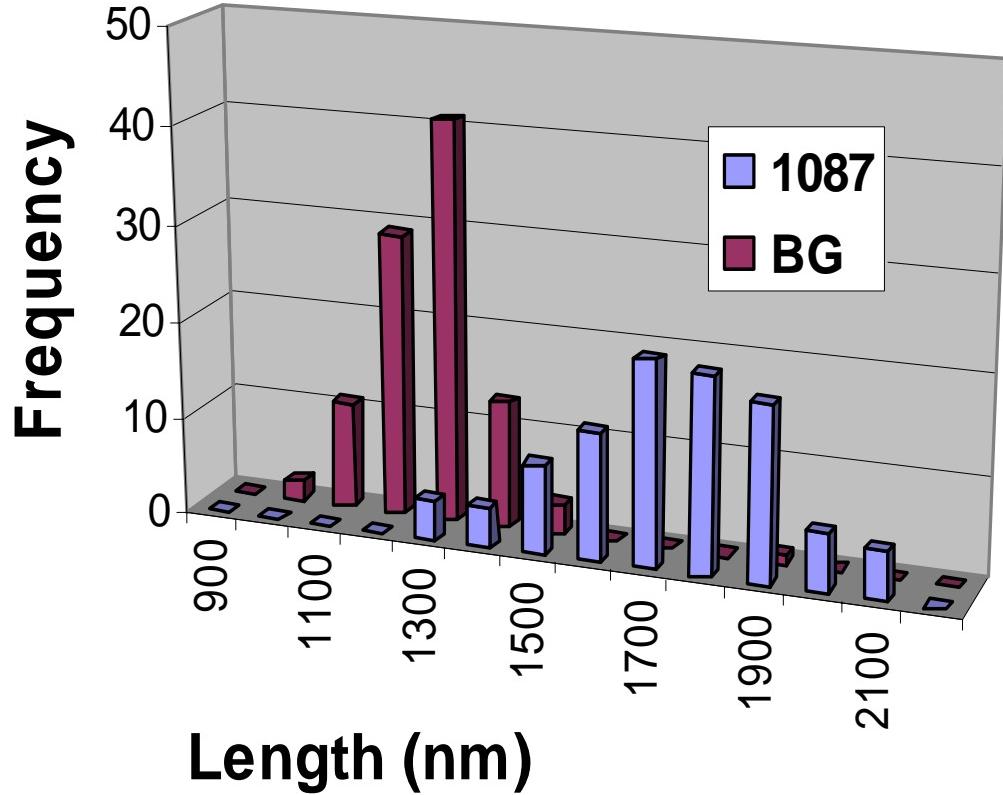
*B. a. NCTC 1087*

Mean **1.91 +/- 0.23**  
Range **1.37-2.45**

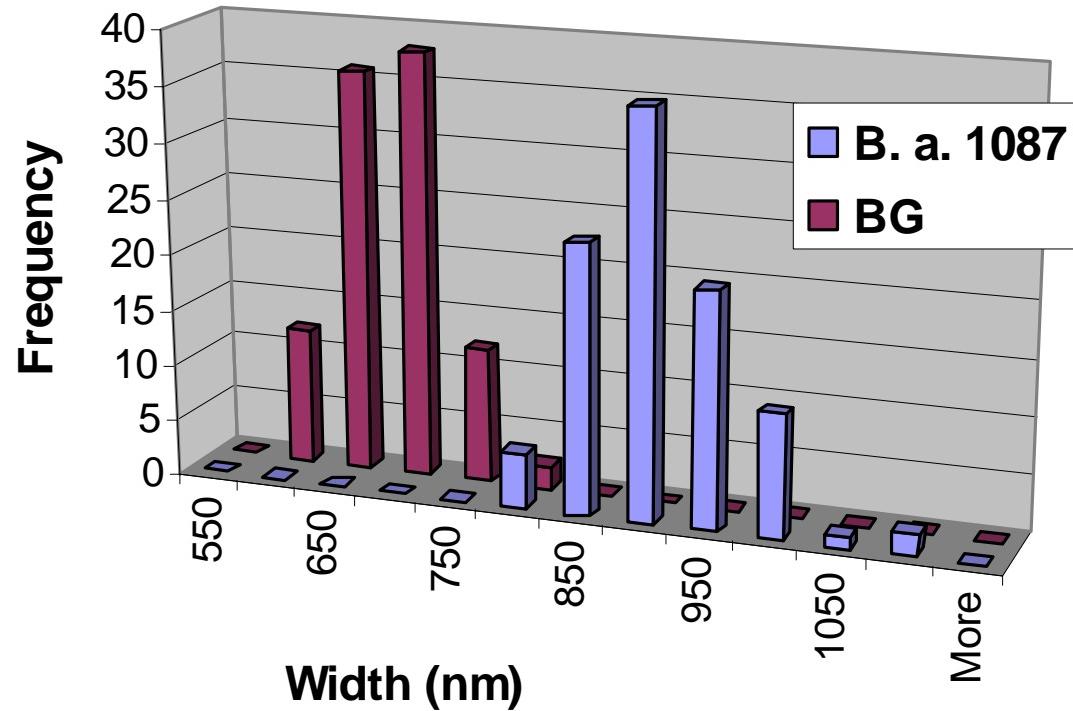
# Comparison of *B. anthracis* with other *Bacillus* species

	Length ( $\mu\text{m}$ )	Width ( $\mu\text{m}$ )
<i>B. anthracis</i> Albia	<b>1.43 +/- 0.14</b>	<b>0.87 +/- 0.07</b>
<i>B. anthracis</i> 1087	<b>1.68 +/- 0.19</b>	<b>0.89 +/- 0.06</b>
<i>B. anthracis</i> Sterne	<b>1.53 +/- 0.15</b>	<b>0.88 +/- 0.06</b>
<i>B. anthracis</i> D-Sterne	<b>1.56 +/- 0.16</b>	<b>0.82 +/- 0.06</b>
<i>B. globigii</i>	<b>1.22 +/- 0.12</b>	<b>0.65 +/- 0.05</b>
<i>B. subtilis</i>	<b>1.07 +/- 0.09</b>	<b>0.48 +/- 0.03</b>
<i>B. cereus</i>	<b>1.55 +/- 0.16</b>	<b>0.90 +/- 0.07</b>
<i>B. megaterium</i>	<b>1.60 +/- 0.16</b>	<b>0.84 +/- 0.07</b>
<i>B. mycoides</i>	<b>1.85 +/- 0.21</b>	<b>0.91 +/- 0.07</b>

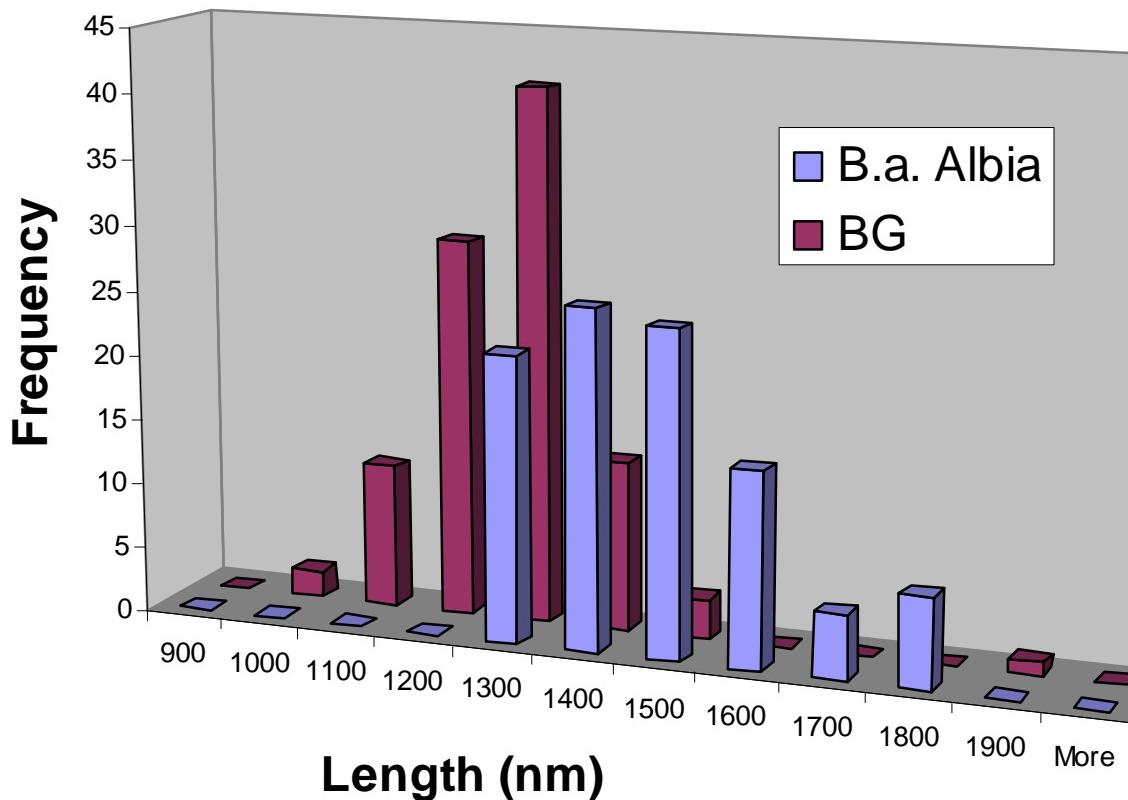
## *B.a. 1087 vs. B. g. (Length)*



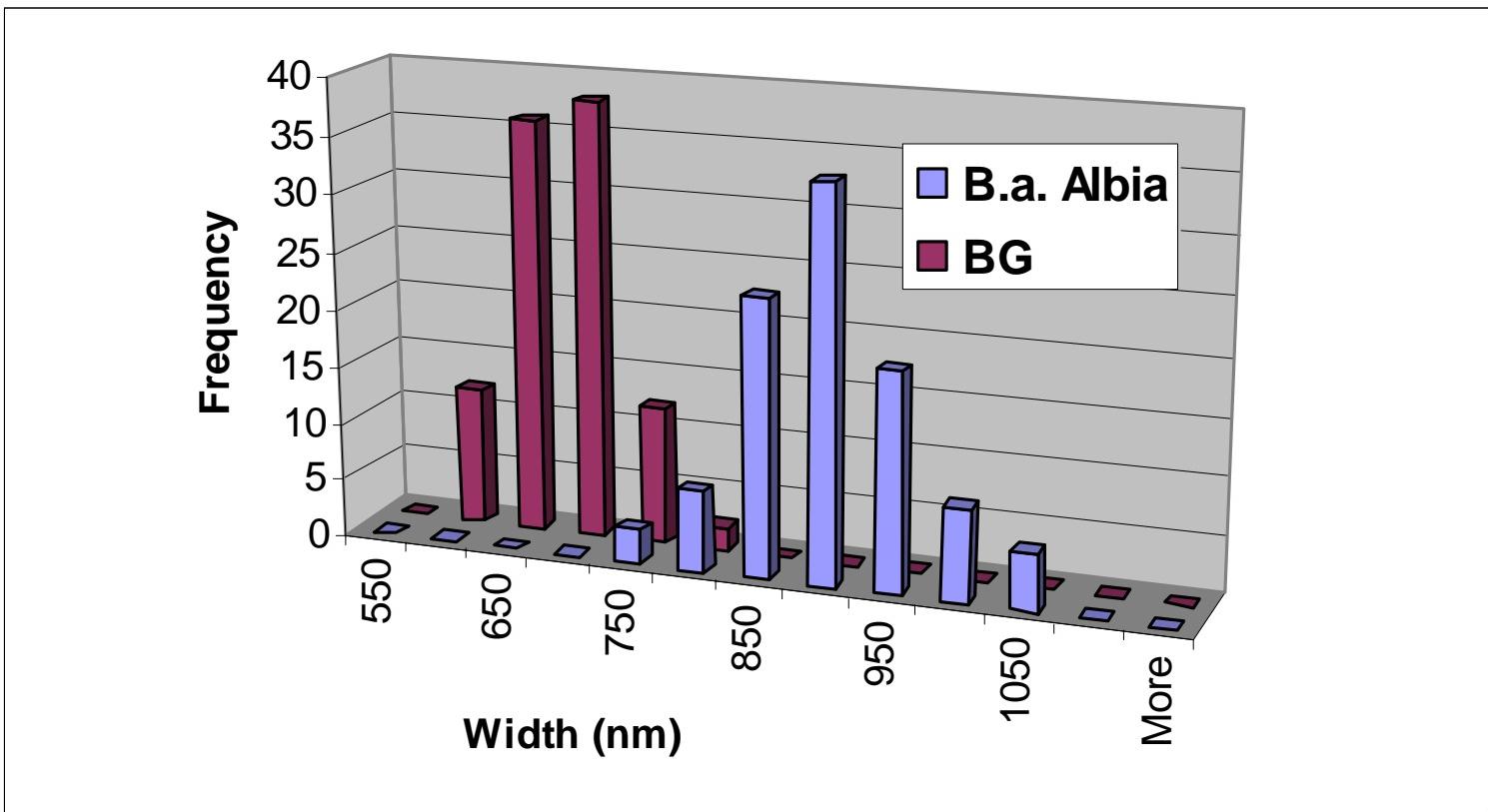
# *B.a. 1087 vs. B. g. (Width)*



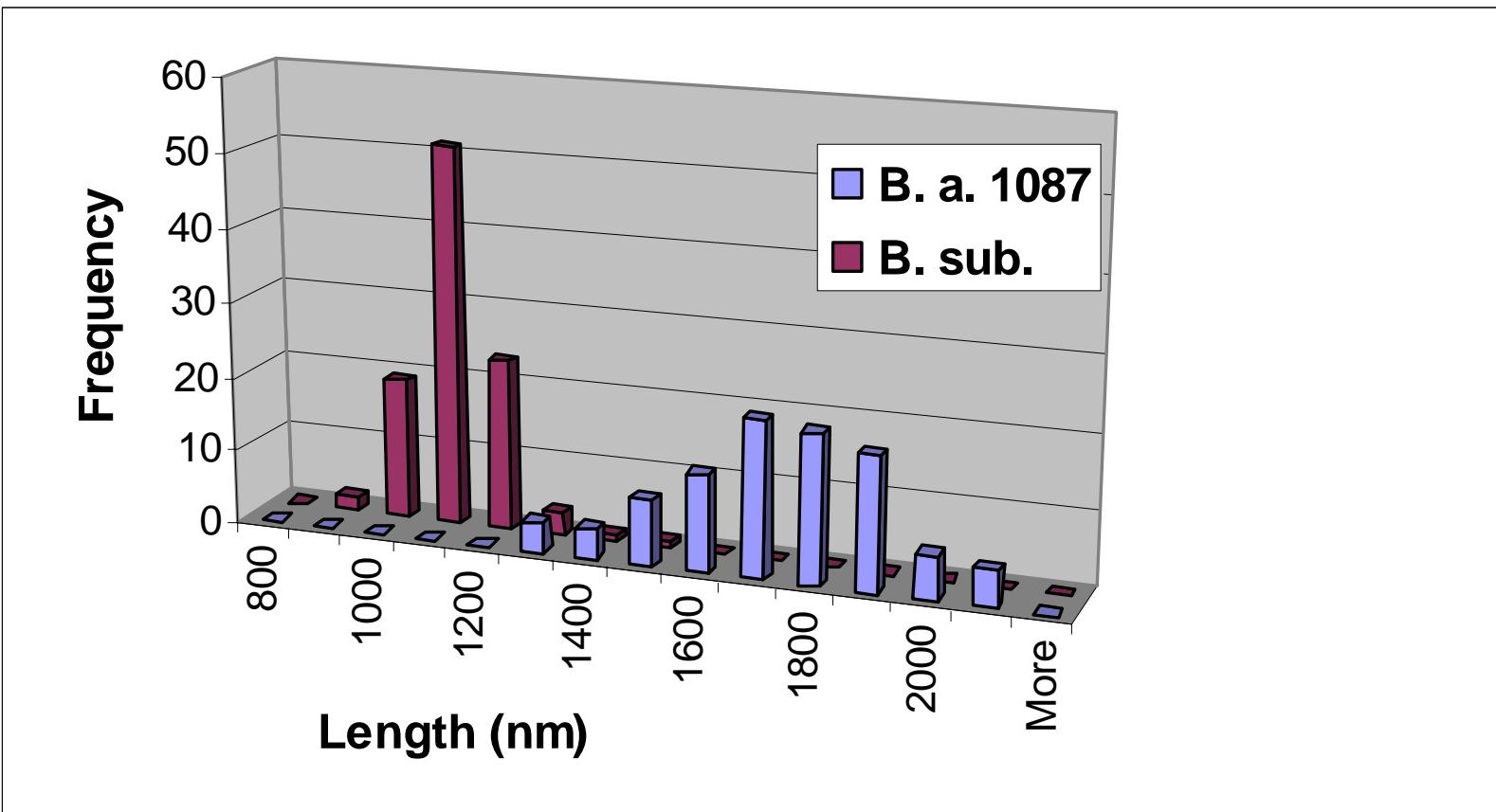
# *B.a. Albia* vs. *B. g.* (Length)



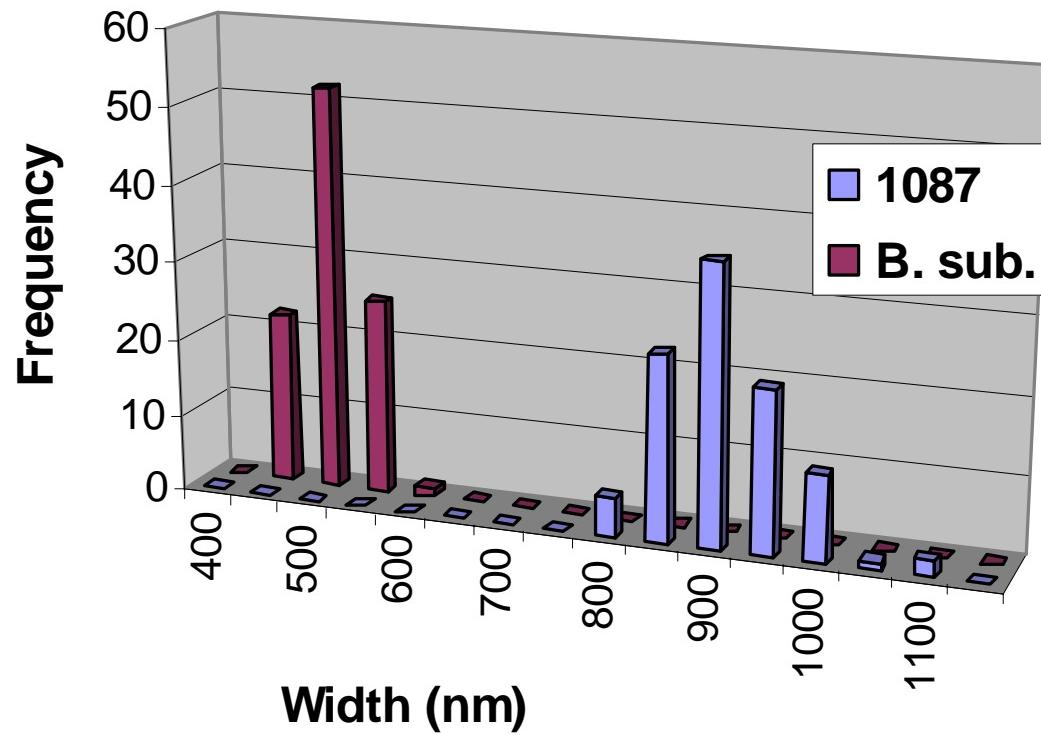
# B.a. Albia vs. B. g. (Width)



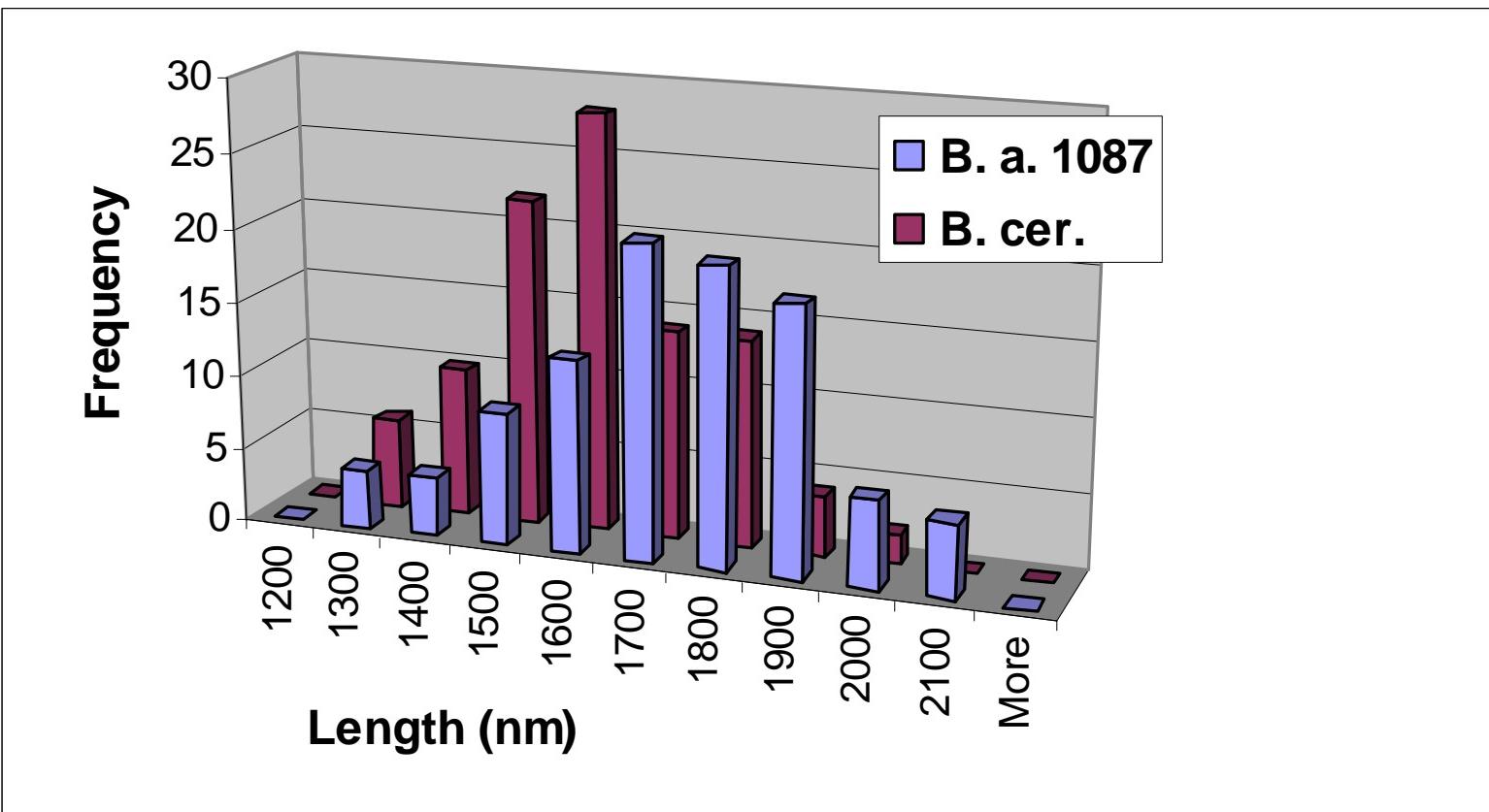
# *B.a. 1087 vs. B. subtilis* (Length)



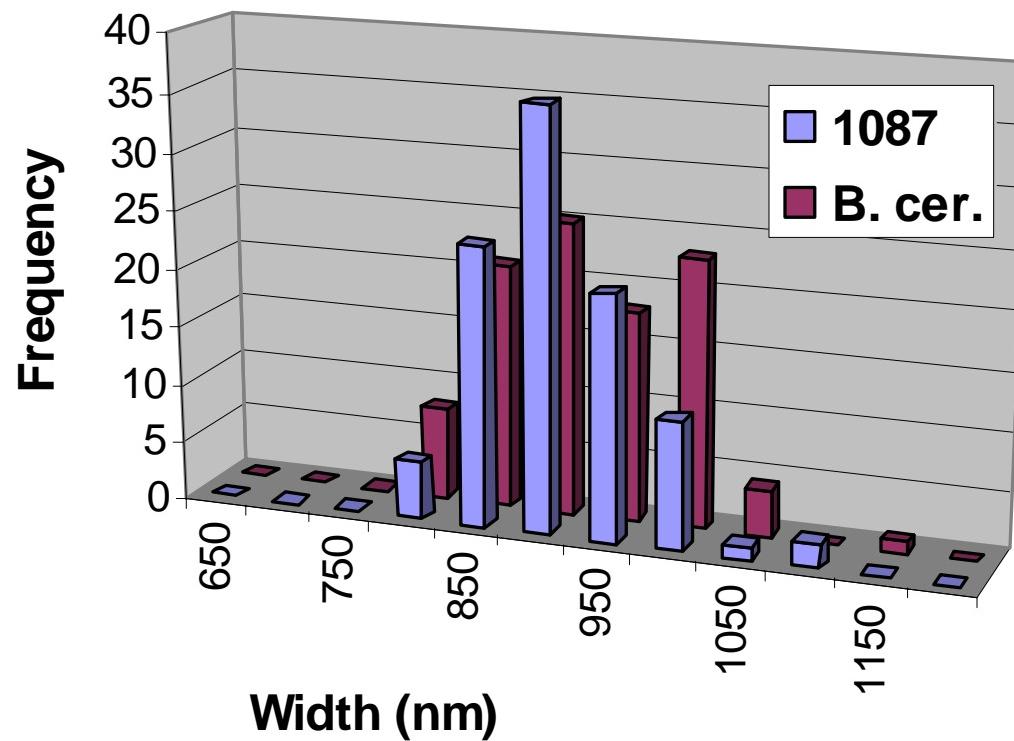
# *B.a. 1087 vs. B. subtilis* (Width)



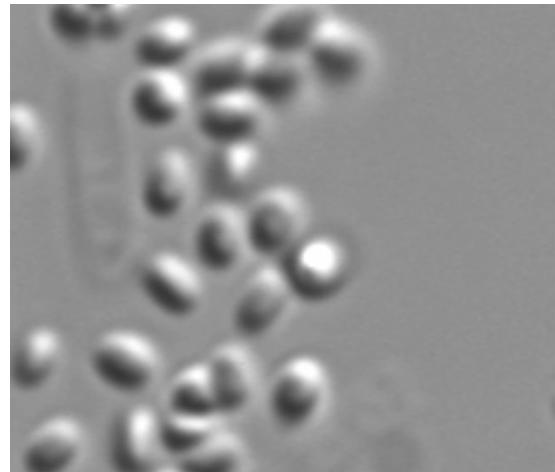
# *B.a. 1087 vs. B. cereus* (Length)



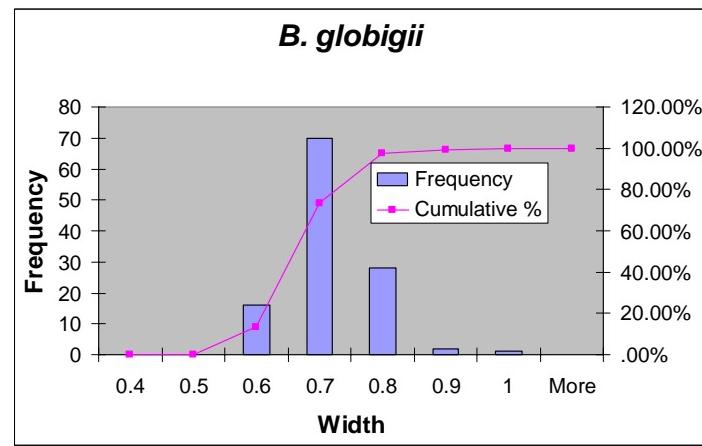
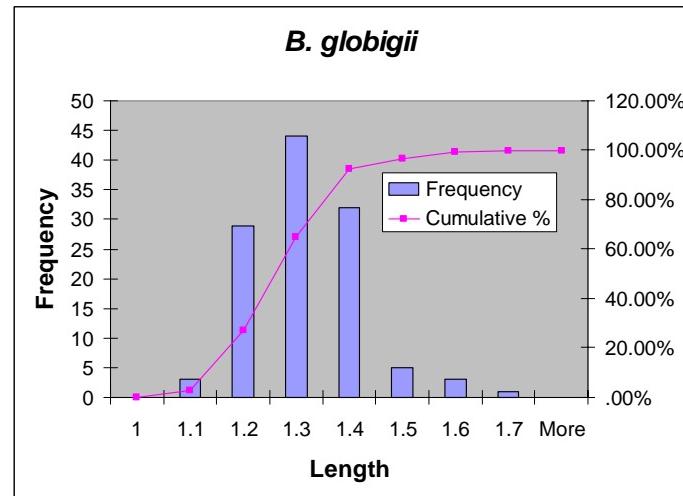
## *B.a. 1087 vs. B. cereus* (Width)



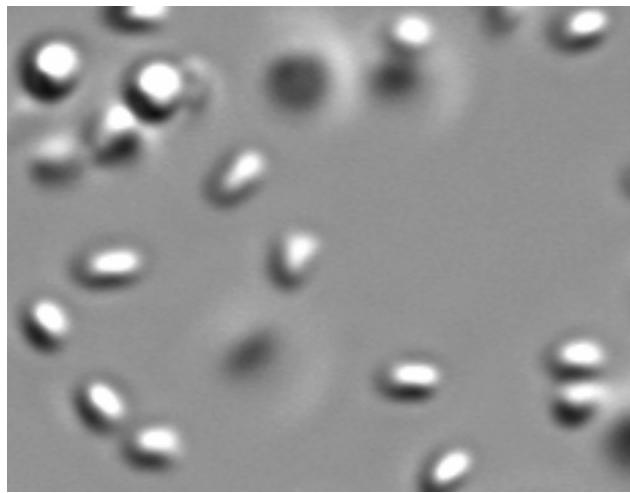
# *B. globigii* SB512 (*B. atrophaeus*)



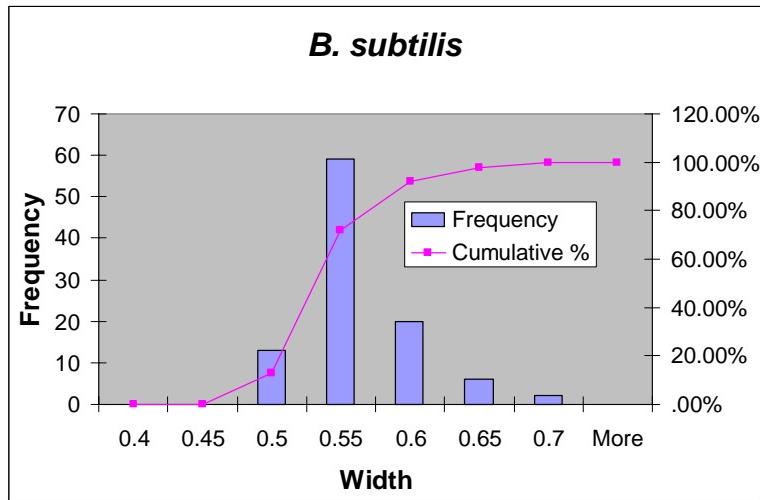
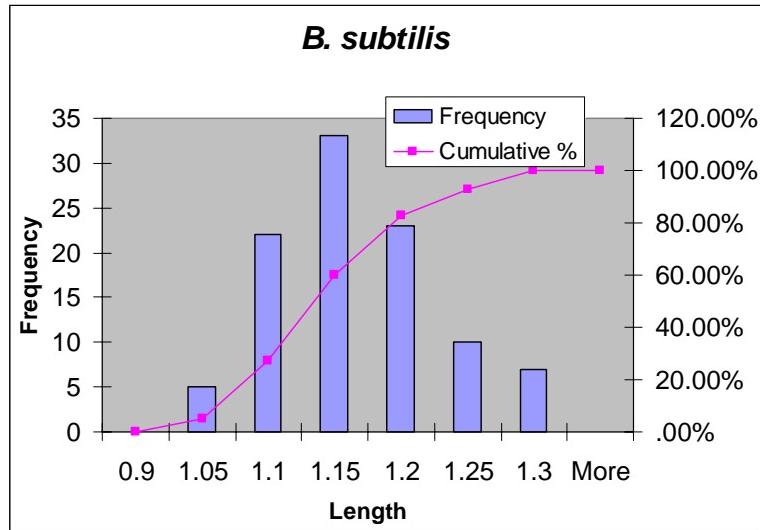
- **Length**  
Mean  $1.27 \pm 0.10 \mu\text{m}$   
Range  $1.04$ - $1.61 \mu\text{m}$
- **Width**  
Mean  $0.67 \pm 0.07 \mu\text{m}$   
Range  $0.50$ - $0.94 \mu\text{m}$



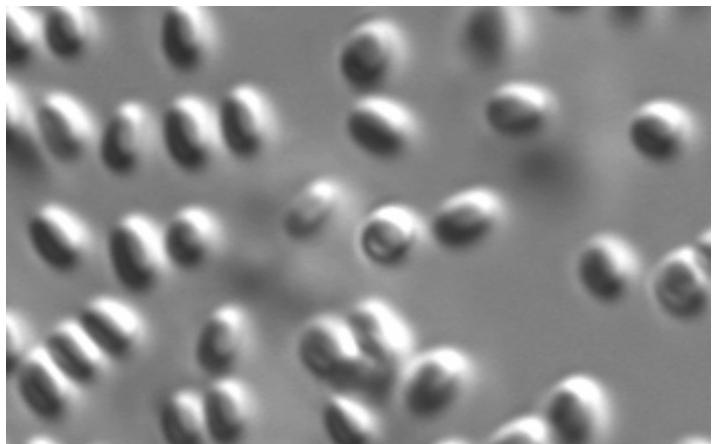
# *B. subtilis* 1031



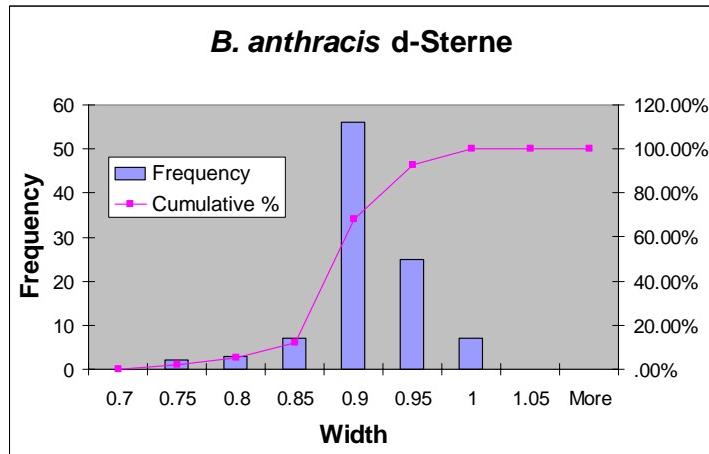
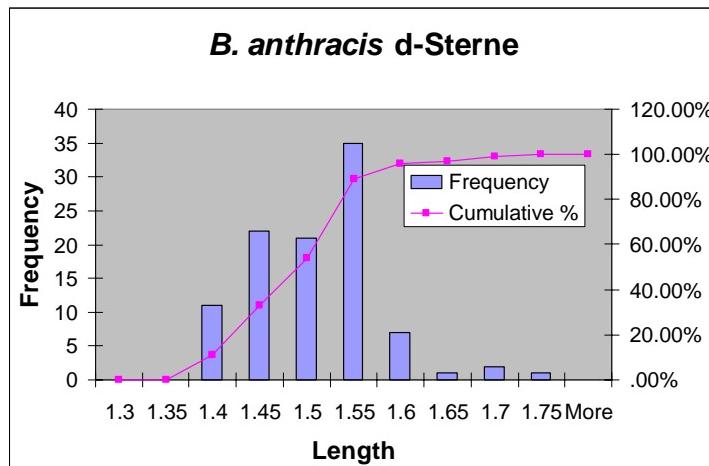
- **Length**  
Mean  $1.14 \pm 0.07 \mu\text{m}$   
Range  $1.04\text{-}1.28 \mu\text{m}$
- **Width**  
Mean  $0.54 \pm 0.04 \mu\text{m}$   
Range  $0.46\text{-}0.69 \mu\text{m}$



# *B. anthracis* ( $\Delta$ -Sterne)



- **Length**  
Mean  $1.48 \pm 0.07 \mu\text{m}$   
Range  $1.37$ - $1.71 \mu\text{m}$
- **Width**  
Mean  $0.89 \pm 0.05 \mu\text{m}$   
Range  $0.75$ - $1.00 \mu\text{m}$



# Comparison of Measurements by TEM vs. OM

			Length	Width
		TEM	1.22 +/- 0.12	0.65 +/- 0.05
<i>B. globigii</i>	OM		1.27 +/- 0.10	0.67 +/- 0.07
	TEM		1.07 +/- 0.09	0.48 +/- 0.03
<i>B. subtilis</i>	OM		1.14 +/- 0.07	0.54 +/- 0.04
	TEM		1.56 +/- 0.16	0.82 +/- 0.06
<i>B. anthracis</i> (d-Sterne)	OM		1.48 +/- 0.07	0.89 +/- 0.05

# CONCLUSIONS

- Systematic comparison of spore properties of several *Bacillus* species.
- Compared spore size properties of 6 species of *Bacillus* by TEM.
- *B. anthracis* spores are significantly larger than spores of the most common simulants, *B. g.* and *B. subtilis*.
- *B. anthracis* spores are similar in size to *B. cereus* and *B. megaterium*.
- Spores of pathogenic and non-pathogenic *B. anthracis* are similar in size.



**ECBC**

# Acknowledgements

## MICROBIOLOGY TEAM

**Leslie Williams**

**Warren Gardner**

## UNIVERSITY OF PENNSYLVANIA – BIOMEDICAL IMAGING CORE LABORATORY

**Neelima Shah**

**Ray Meade**



**ECBC**